Effect of elementals inside *Bacopa monnieri* herb due to acid attack

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Abstract: - In the present investigation, the main motivation was to study the sulphuric-simulated acid rain (S-SiAR) induced effect in the Brahmi (Scientific name *Bacopa monnieri*) using particle-induced X-ray emission (PIXE) technique. It is observed that, the concentration of minor elements viz. Fe, Ca, K, Cl, etc., and trace elements viz. V, Cr, Mn, Cu, Zn, etc. found to be different due to acid induction. The pH analysis shows that the acidic properties of *B. monnieri* (pH = 5.27± 0.02) extract decreased after being exposed to acid rain, i.e., the acidity decreases due to AR i.e., the acidity decreases due to AR (increase of pH value). Instead of acid rain-induced acidity in the plant, *B. monnieri* herb shows a decrease in acidity due to acid induction. It is expected that various important medicinal properties of *B. monnieri* viz. memory-boosting mechanism can be drastically because of a decrease in its acidic properties and formation of new complexes inside, which may have a bad effect on the user's health.

Key-Words: - Medicinal herb, Sulphuric-simulated acid rain (S-SiAR), Elemental analysis, *B. monnieri*

Received: June 19, 2022. Revised: August 4, 2023. Accepted: September 4, 2023. Published: October 2, 2023.

1 Introduction

Particle-induced X-ray emission (PIXE) is an accelerator-based elemental detection technique widely used for elemental identification [1-3]. Elements in a sample can be analysed by applying this technique limited to $Z > 12$. The PIXE technique plays an important role in the estimation of trace, minor, and major elemental composition present in various biological (plants and animals) systems. The existence of various vital elements present in the medicinal herb and its usefulness for the treatment of different kinds of disease.

Acid rain (AR) can have harmful effects on plants, aquatic animals, humans, and infrastructure. Rainwater having pH < 5.6 and ion concentration of $H^+ > 2.5\ \mu eq^{-1}$ is considered to be acidic [4]. The concentrations of both primary (SO_2 and NO_x) and secondary (O_3) pollutants are mainly responsible for the acidic nature of the AR. The medicinal herbs are drastically affected by AR [5-10]. Keeping the above objective aspects in view the medicinal herb *Bacopa monnieri* (English name *Brahmi*, Family: *Scrophulariaceae*; Genus: *Bacopa*; Species: *B. monnieri*) as shown in Fig. 1, has been selected for the present studies. The motivation behind this study was to investigate the sulphuric-simulated acid rain (S-SiAR)-induced effects in the *B. monnieri* herb using pH and PIXE techniques, and to find out the resultant changes of properties of the AR-induced medicinal herb.

Fig. 1 A flowering stem of the *Brahmi* plant

2 Experimental

The S-SiAR-induced effect on medicinal herbs like *B. monnieri* was analysed using the PIXE technique. The medicinal herb *B. monnieri* was treated with S-SiAR of pH about 4 for 22 weeks on the campus of the Institute of Physics (IOP), Bhubaneswar. A handy pH tester (OAKTON pH Tester-20, EUTECH Instruments) was used for the measurement of the pH of the extract of the above experimental samples. For convenience and easy handling of the acid-treatment process on the herbs every day, a special type of syringe (least measuring unit 0.1 ml) which is non-reactive to chemicals was used. An amount of 0.1 ml (one drop) of 10% dil. H_2SO_4 in the tap water of volumes 450 ml, 700 ml, 850 ml, and 1100 ml produces an acidic solution of pH 3.39, 4.14, 5.10, and 5.45, respectively. These fractional pH values so prepared are in the suitable range of H_2SO_4-based acid rain observed in nature. Fresh acidic solutions were prepared every day to obtain the numerical stability of the above solution used in the simulation work. Analysis of both controlled (450 ml of normal water) and S-SiAR treated (450 ml of acidic water) *B. monnieri* samples were carried out applying the PIXE technique using 3MeV proton generated from an accelerator (3MV Tandem pelletron, National Electrostatic Corporation, USA) at the Institute of Physics (IOP), Bhubaneswar. The proton beam was bombarded on the solid pallet prepared from the dry *Brahmi* herb to get the X-ray spectra of each element present in the sample.

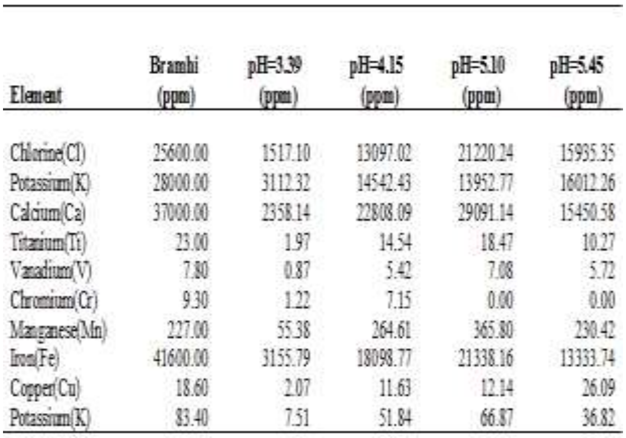
3 Results and Discussion

The PIXE spectra of all the treated and untreated *B. monnieri* were recorded using the calibrated multichannel analyser (MCA) with Am^{241} X-ray source. The recorded spectrum of both controlled and S-SiAR-treated *B. monnieri* is presented in Fig. 2. The profile fitting (non-linear least squares) of each individual spectrum was carried out using GUPIX- 2000 software [1-3, 11]. Both quantitative and qualitative estimations of the elements present and concentration were carried out. Experimental parameters viz. the Energy of the incident beam, the net charge collected, the window thickness of the chamber, the Si(Li) detector parameters, and the experimental geometry were taken into consideration in the above calculation.

The PIXE data with their comparison are presented. The minor and trace elements present in all the *B. monnieri* samples have been detected and the respective concentrations are tabulated in Table 1. A number of minor elements viz. Fe, Ca, K, Cl, etc. were observed. Similarly, the trace elements in increasing order of concentration i.e., V, Cr, Mn, Cu, Zn, etc. were observed. In the herb growing process, normal tap water (pH = 6.29-6.85, at 27 ºC) was used for the growth of the control group of the herb. The raw *B. monnieri* extract (stem and leaf juice, 5 ml) was found to be acidic with pH = 5.27 ± 0.02 (at 27 ºC). Hence, the ionic $[H^+]$ concentration in the above normal herb extract (5 ml) has been calculated to be 5.37×10^{-6}, applying the relation $10^{-pH} = [H^+]$, or $pH = -\log [H^+]$.

Similarly, the concentrations of $[H^+]$ in the S-SiAR acidified solutions were estimated to be 3.55×10^{-6} (pH = 5.45), 7.9×10^{-6} (pH = 5.10), 7.24×10^{-5} (pH = 4.15), and 4.07×10^{-4} (pH = 3.39).

Table 1 PIXE data of various elements obtained from control and S-SiAR effected *Bacopa monnieri*

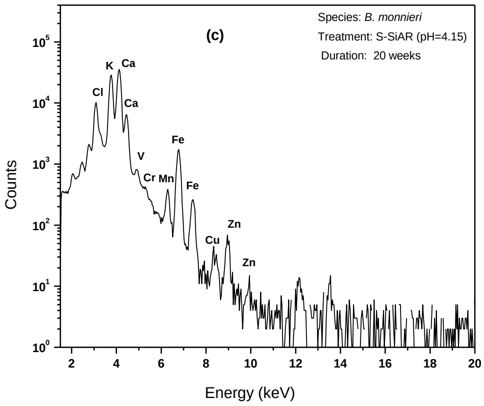


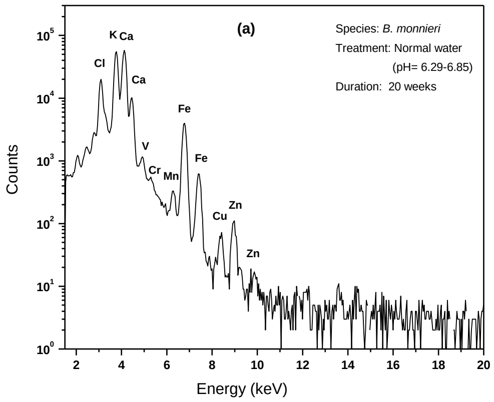
Element	Brambi (ppm)	pH=3.39 (ppm)	pH=4.15 (ppm)	pH=5.10 (ppm)	pH=5.45 (ppm)
Chlorine(Cl)	25600.00	1517.10	13097.02	21220.24	15935.35
Potassium(K)	28000.00	3112.32	14542.43	13952.77	16012.26
Calcium(Ca)	37000.00	2358.14	22808.09	29091.14	15450.58
Titanium(Ti)	23.00	1.97	14.54	18.47	10.27
Vanadium(V)	7.80	0.87	5.42	7.08	5.72
Chromium(Cr)	9.30	1.22	7.15	0.00	0.00
Manganese(Mn)	227.00	55.38	264.61	365.80	230.42
Iron(Fe)	41600.00	3155.79	18098.77	21338.16	13333.74
Copper(Cu)	18.60	2.07	11.63	12.14	26.09
Potassium(K)	83.40	7.51	51.84	66.87	36.82

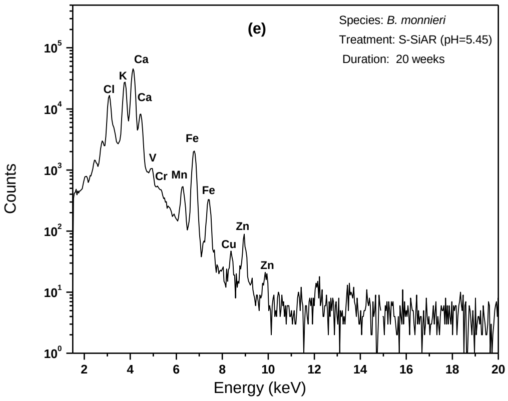
1%=10, 000 ppm = 10 mg/g

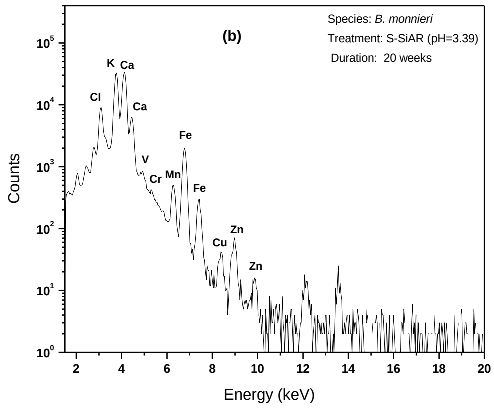
Minor elements: Ca, K, Cl, Fe: ±1-2% ; Other elements: ±10-12%

The strength (concentration, C) of the unit (1ml) controlled *B. monnieri* extract is around 7.67×10^{-4} M. Again, the degree of dissociation α, and dissociation (ionization) constant K_a are calculated to be 7.001×10^{-3} and 3.76×10^{-8}, respectively. Keeping the amount of water fixed (5 ml) and increasing the volume of extract, it was found that the pH value gradually moved towards the normal value. The reason is that by keeping the volume of water ($[H^+] = [OH^-] = 10^{-7}$) fixed and increasing the amount of the extract ($[H^+] = 10^{-5.27}$), the pH of the mixture increases. The degree of acidity is measured by pH value, which is a shorthand version of potential hydrogen. Actually, the degree of acidification is the pH of the water, which is defined as the negative logarithm of the concentration of hydrogen ion, i.e., $pH = -\log [H^+]$. By plotting a calibration curve, i.e. Fig. 3 between the volume of extract vs. pH at a constant amount of water 5 ml, it is observed that at 500 μl of extract, the diluted solution shows the pH value the same as the pH of the controlled *Brahmi*. So, on diluting 50 μl extract (controlled *Brahmi*) with 5 ml of distilled water (pH = 7.05± 0.05), it becomes highly acidic (pH = 2.88). The pH values (or H$^+$ concentration) of the S-SiAR treated species (*B. monnieri*) after diluting [extract (500 μl) + water (5 ml)] were found to be 5.54 (= 2.88×10^{-6}), 5.57(= 2.69×10^{-6}), 5.62(= 2.60×10^{-6}), and 5.59 (= 2.57×10^{-6}).









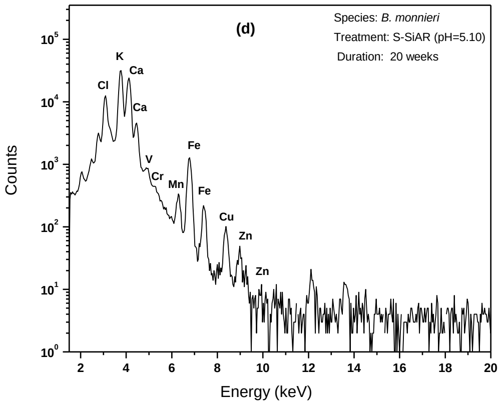


Fig. 2 PIXE spectrum of control (a) and sulphuric-simulated acid rain affected (b-e) *Bacopa monnieri* herb (stem and leaves)

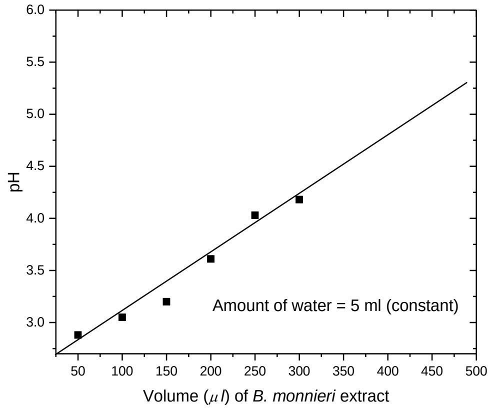


Fig. 3 Calibration plot showing variation of pH on dilution of *B. monnieri* extract

The average pH value of the S-SiAR treated plant materials (*B. monnieri* extract of five samples) was found to be 5.62 ± 0.02. It is interesting to note that the acidic properties of *B. monnieri* extract decreased after being exposed to acid rain, i.e., the acidity decreases due to AR. Instead of acid rain-induced acidity in the plant, *B. monnieri* herb shows a decrease in acidity due to acid induction as observed earlier using energy dispersive spectroscopy [5]. The result indicates that the medicinal herb loses its acidic property after being exposed to S-SiAR. The pH results measured with an accuracy of ± 0.02, with a maximum uncertainty is about 1-1.5%.

The proton induced X-ray yield, Y(z)The characteristic X-ray yield, $Y(z)$ of an element (Z) can be expressed as $Y(z) = Y'(z)\,[H\,Q]\,[C_z\ \varepsilon_z\ t_z]$, where the symbols $Y'(z)$, H, Q, C_z, ε_z and t_z are the computed theoretical yield from the database (per steradian per unit concentration), the instrumental constant, the total beam charge, the concentration of the element in a given target matrix per unit integrated beam charge, the efficiency of the X-ray detector, and the penetrating thickness of X-rays in the air medium between detector-sample respectively [12].

The concentrations of an unknown elemental can be obtained by) applying the relation $\frac{Y_{samp}}{Y_{std}} = \frac{C_{samp}}{C_{std}} \cdot \frac{S(E)_{std}}{S(E)_{samp}}$, where *Y*, *C*, and *S(E)* are the yield, concentration and stopping power of the sample and standard (or reference material), respectively [13]. The minor and trace elements play an important role by possessing different curative capabilities for human diseases. Due to limitations of the detector, geometry, and vacuum incompatibility, elements of atomic number greater than 16, i.e., Z>16 were detected using the present PIXE setup. The element Fe concentration

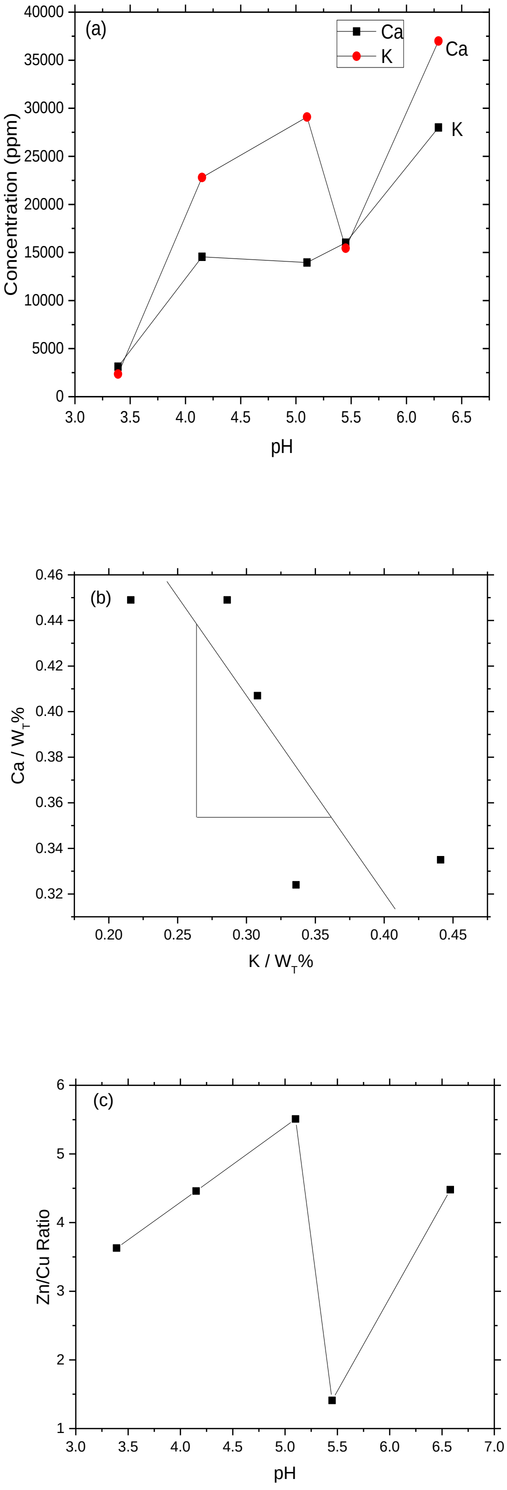


Fig. 4 Interrelationship between elements present using PIXE: (a) pH vs. Ca-K, and (b) K/W_T% vs. Ca/W_T%, and (c) pH vs. Zn/Cu Ratio

measured by the PIXE technique was found to be quite high as compared to the result obtained using the EDS technique [5], which may be due to contamination of X-ray lines, while passing through the steel (Fe) slit. We suspect that the extra contribution is due to the Fe X-ray photons emitted from the steel slit excited by higher-energy X-ray photons emitted by the sample. Hence, the Fe concentration was not taken into consideration while estimating the other elemental concentration. Again, in an earlier report high value of iron about 15743.7± 1075.5 ppm was observed in the soil of the IOP campus, where the experimental plants were grown [14]. Since elemental concentration of soil and the effect of soil acidity are responsible for the uptake of the traces by the plants [15].

Among the above minor elements, K and Ca play a major role in various biological activities and form salts after interacting with acid rain. The plot pH vs. concentration of Ca and K interrelationship shows that the acid interaction is enhanced after the particular pH around 5.5, which is nearly the same as the pH of the herb material, as shown in Fig. 4(a). Again, the plot K/W_T% vs. Ca/W_T% (where W_T% in mg/g is the total weight percentage after subtracting the Fe amount since the contamination line is mixed with the actual concentration), as shown in Fig. 4(b), possesses a negative slope (= -0.857) which indicates that the concentration of K is inversely proportional to that of Ca and vice versa. In other words, due to the interaction of S-SiAR with the elements such as K and Ca present in the herb material, their concentration can be affected in an inverse proportion with each other in the acidic environment. It is interesting to note that the acidic properties of *B. monnieri* extract decreased after being exposed to acid rain, i.e., the acidity decreases due to AR. Instead of acid rain-induced acidity in the plant, *B. monnieri* herb shows a decrease in acidity due to its self-neutralizing behavior [5].

Again, trace elements such as Cu and Zn are essential constituents in almost all biochemical processes and the Zn/Cu ratio is found to be well correlated in our study as shown in Fig. 4(c). The elemental concentration of Cu and Zn shows very peculiar behavior after being treated with S-SiAR. Because of the interaction of acid rain of the lowest pH (3.39) with *B. monnieri* herb the metals are ionized, the formed cations are liberated, and can be rapidly leached out from the herb. The stronger the acid rain (pH 3.39) leaching is more and element Cu of the lowest concentration was observed. By decreasing the strength of acid rain (increasing pH) the Cu concentration increases up to a certain value (5.27) for the system. Since pH of the *in-vivo* grown or controlled *B. monnieri* extract (acidic) is 5.27.

The Cu concentration is maximum (18 ppm) for the controlled *B. monnieri*. Weaker the acid rain (pH 5.45) leaching is less and the element Cu of the highest concentration was observed. Since the pH of acid rain is higher with respect to the pH of controlled *B. monnieri* extract (5.27). Considering the controlled *B. monnieri* herb as neutral, the pH 5.45 acts as alkaline with respect to its pH. Again, Cu concentration increases in alkaline solution [16]. Hence, the concentration of Cu in *B. monnieri* observed at 5.45 is 26 ppm (more) with respect to the control (18 ppm). Similarly, the Zn concentration is maximum (83 ppm) for the in-vivo grown *B. monnieri* (control). Because of the interaction of acid rain of the lowest pH (3.39) or more acidic with *B. monnieri* herb, the metals are ionized, and the formed cations are liberated, and can be rapidly leached out from the herb. The stronger the acid rain (pH 3.39) leaching is more in an element Zn of the lowest concentration was observed. Similarly, by decreasing the strength of acid rain (increasing pH) the Zn concentration increases up to a certain value (5.27) for the system. Since the pH of the controlled *B. monnieri* extract (acidic) is 5.27. Considering the controlled *B. monnieri* herb as neutral, the pH 5.45 acts as alkaline with respect to the *B. monnieri* (controlled). Again, Zn concentration is reduced due to the lower solubility of Zn in alkaline solution [17]. Hence, the concentration of Zn in *B. monnieri* observed at 5.45 is 37 ppm (less) with respect to the controlled BM (83 ppm). Similarly, other elements possess peculiar behavior because of the acidic nature of *B. monnieri* extract and induction of S-SiAR.

The S-SiAR-treated medicinal herbs like *B. monnieri* may lose their vital properties like memory-boosting mechanism. In a separate report, it has been reported that the pollutants of acid rain cause the formation of toxic aluminum salts viz. $Al_2(SO_4)_3$, in the different parts of *B. monnieri* herb [18]. It has been well documented that salt ($Al_2(SO_4)_3$) toxicity causes a number of health problems.

The errors in the above quantitative analysis of the elemental concentration is because of the error in the measurement of peak area, H-value, and beam charge associated with this quantification. The uncertainty in the estimation of the concentration of the major elements was found to be in the range of

1– 2%, whereas for the minor and trace elements was found to be between 10–12%.

4 Conclusion

In conclusion, the effect of sulphuric-simulated acid rain on the concentration of elements viz. Fe, Ca, K, Cl, Mn, Zn, Ti, Cu, Cr, V, etc. existing in the herb *B. monnieri* have the major attention of the present investigation both for its pharmaceutical interest and for important environmental issues. Therefore, we expect that the various important medicinal properties of *B. monnieri* are drastically affected because of a decrease in its acidic properties and the formation of new complexes inside, which may have a bad effect on the user's health. Regarding the practical application of the above study, this insists that the medicinal herb grown in the acid rainfall area must check for its elemental constituents and compositional phases by applying various X-ray techniques viz. PIXE, XRD, etc., for the confirmation of the existence of toxic elements or phases, if any before processing for its pharmaceutical/medicinal applications. The same can be applicable to other medicinal herbs and vegetable plants. The formation of toxic salts like $Al_2(SO_4)_3$ in medicinal plants and vegetable plants grown in the acid rainfall area is also expected. This is a good indication related to the physical protectiveness of *B. monnieri* towards acid rain. However, the S-SiAR-induced biological effects, cellular effects, etc. cannot be ignored. Hence the study is important to understand the AR-induced leaching caused effects and defects in plants in general and medicinal plant *B. monnieri* in particular.

Acknowledgement:
Authors would deeply acknowledge Professor P. C. Mishra, Ex-professor and Head of the Department of Environmental Science, Sambalpur University, India, and Professor T. N. Tiwari, Ex-professor and Head of the Department of Physics, NIT Rourkela, India for their timely help, valuable suggestions and encouragement to carry out the study.

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Contribution of Individual Authors

The authors equally contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

Sources of Funding

No funding was received for conducting this study.

Conflict of Interest

The authors have no conflicts of interest relevant to the content of this article.

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